RESPIRATION RATES IN PLANARIANS. III. THE EFFECT OF THYROID COMPOUNDS ON OXYGEN CONSUMPTION[1]

MARIE M. JENKINS

Department of Zoology, University of Oklahoma, Norman, Oklahoma

Iodinated proteins have been found throughout the invertebrate world (Roche, 1952; Gorbman *et al.*, 1954), primarily in the form of mono- and diiodotyrosine, although in a number of insects (Limpel and Casida, 1957) and in *Musculium*, a fresh-water fingernail clam (Gorbman *et al.*, 1954), a high percentage of the protein-bound iodine has been shown to be in the form of thyroxine. None of these compounds has been demonstrated unequivocally to take part in physiological processes in the invertebrate animal (Goldsmith, 1949; Gorbman *et al.*, 1954), but recent reports indicate the question is not settled. Wingo and Cameron (1952) found that thyroxine hampered the multiplication of a ciliate protozoan, *Tetrahymena geleii*, but increased the rate of oxygen uptake above that of parallel control cultures. Thyroxine added to the diet of rice moth (*Corcyra cephalonica*) larvae is reported to have increased the oxygen consumption requirement, although thyroglobulin was without effect (Srinivasan *et al.*, 1955).

The presence of iodinated proteins in planarians has not been investigated, but several workers have reported a positive action of thyroid compounds on physiological activities in this group. Castle (1928) observed that *Phagocata* (*Planaria*) *velata* was attracted to and fed readily upon macerated sheep thyroid, and subsequently decreased in size even more rapidly than worms subjected to starvation. Goldsmith (1937), studying the effect of endocrine feeding on regeneration and growth in *Dugesia tigrina* (*Planaria maculata*), noted no significant differences in the head regeneration time in the gland-fed animals, but found that thyroid-fed individuals increased in size to a lesser extent than the liver- and pituitary-fed forms. The influence of thyroxine on eye formation in *Phagocata gracilis* was investigated (Weimer *et al.*, 1938) in pieces of planarians cut at different levels and allowed to regenerate in a saturated thyroxine solution. Once the reconstitution process had begun, the rate of eye formation was reported to be much higher for the pieces in thyroxine.

No reports are available of the effect of thyroid hormones on oxygen consumption in planarians. Phenylthiourea, an anti-thyroid agent, has been shown, however, to exert a depressing effect on planarian respiration (Jenkins, 1961). In view of these findings an investigation was undertaken to ascertain the effect of certain thyroid compounds on respiration rates in *Dugesia dorotocephala*, a common fresh-water planarian.

[1] Supported in part by grants from the National Science Foundation (G–3209), the Southern Fellowship Fund, and the University of Oklahoma Alumni Development Fund. This study represents part of a dissertation submitted in partial fulfillment of the requirements for the Ph.D. degree at the University of Oklahoma, under the direction of Dr. Harriet Harvey.

Design of Experiment

The planarians used in this study were large, sexually mature animals, collected from Buckhorn Springs[2] in Murray County, Oklahoma. They were maintained in pans of lake water, provided with an aerator, at a constant temperature of 20° C. Experimental animals were taken on the seventh day after feeding and were not fed during the course of the experiment.

Compounds used for this investigation were thyroxine (T_4), 3,5,3′-triiodothyronine (T_3),[3] and 3,5-diiodotyrosine (DIT). In order to determine the concentration to be used, groups of cut posterior ends of planarians were allowed to regenerate in a graded series of molar solutions of each of the compounds in lake water, and the regenerated animals examined for signs of any abnormalities. In worms in both the thyronines, eye spots were visible under a dissecting microscope by the third day, compared to the fifth day for the animals in water and in diiodotyrosine. It was noted, however, that the worms in the 3×10^{-5} *M* dilution of triiodothyronine appeared to have a slight thickening across the head behind the eyes. This was not apparent in the 2×10^{-5} *M* solution; the latter was therefore chosen as the higher concentration for the experiment.

All three chemicals were made up at this concentration so their effects could be compared. In addition, a solution of half the molarity given above was used for each in order to test whether or not a more dilute solution would have an appreciable physiological effect. The controls were cultured in lake water.

The procedure was similar to that employed for observing the effect of goitrogens on oxygen consumption in planarians (Jenkins, 1961) with the following exceptions: The Latin square method was used in order to provide maximum randomization. Seven replicate experiments were performed. Each replicate experiment employed seven groups of five planarians each; one group for each of the two concentrations of the three chemicals used, and one water control. On the day that a replicate experiment was begun, 35 of the largest specimens in one stock pan were selected and divided randomly into the seven unit groups.

For each replicate experiment, oxygen consumption determinations were made as follows:

Day 0: Oxygen consumption was measured over a period of three hours with all seven groups of animals in water. At the close of the day's readings, each group was placed in an individual fingerbowl of water until the following day.

Day 1: The Warburg flasks were prepared with the solutions of thyroid compounds (or of culture water) to be used. The planarians were placed in the flasks and manometer readings were made during the first 3½ hours of exposure to the chemicals. At the termination of the day's readings, each group was placed in a fingerbowl containing the same concentration of thyroid compound as that in which oxygen consumption was to be determined during the remainder of the experiment.

Further readings for periods of three hours were made on the second, fourth, and sixth days. The worms were placed in fresh thyroid compound solutions every second day.

[2] Acknowledgment and thanks are due to Oscar Lowrance, owner of Buckhorn Springs property, for permission to collect the planarians.

[3] Supplied through the courtesy of the Sigma Chemical Company, St. Louis, Missouri.

Results

The oxygen consumption of each group of planarians was calculated according to standard methods (Umbreit *et al.*, 1949) and the results are given in Table I. Little variation was shown among the groups in response to treatment on any one day, the normally-occurring slight downward trend shown by the controls being apparent in each of the experimental groups. The data show significantly that,

Table I

Effect of thyroid compounds on oxygen consumption of planarians

Treatments	μl. O_2/gm./hr.				
	Days				
	0	1	2	4	6
Water	130	128	131	120	121
Thyroxine, 2×10^{-5} *M*	133	130	130	121	120
Thyroxine, 1×10^{-5} *M*	132	129	131	120	124
Triiodothyronine, 2×10^{-5} *M*	129	133	128	124	124
Triiodothyronine, 1×10^{-5} *M*	131	130	131	121	120
Diiodotyrosine, 2×10^{-5} *M*	133	132	128	123	119
Diiodotyrosine, 1×10^{-5} *M*	126	130	130	124	125

under the conditions of this experiment, there is no demonstrable effect on the oxygen consumption of the planarians.

Discussion

Although neither iodotyrosines nor iodothyronines, which are widely distributed among invertebrates, have been found to influence physiological processes in these animals, it is obvious that there is some homeostatic regulation in organisms without a thyroid, and that metabolic processes do occur and are regulated within the animal. Whether thyroxine or its analogs play any part in this regulation remains to be established. The prevailing opinion at the present time is that invertebrate tissues are insensitive to the action of thyroid hormones. Findings which are not in agreement, such as moth larvae showing an increased metabolic rate when fed with thyroxine (Srinivasan *et al.*, 1955), have not been confirmed in other invertebrates.

Although evidence of the physiological activity of thyroid hormones in metazoan invertebrates is inconclusive, there is increasing evidence that one-celled organisms respond markedly to these chemicals. The findings of Wingo and Cameron in regard to *Tetrahymena geleii* have been mentioned above. Gutenstein and Marx (1957) have demonstrated that respiration of yeast cells (*Saccharomyces cerevisiae*) is significantly accelerated by thyroxine and inhibited by a specific thyroxine antagonist. Augmentation of oxygen consumption has also been observed in *Escherichia coli* subjected to the influence of T_3 and T_4 (Roche *et al.*, 1959), although the respiratory action of the two iodothyronines does not seem bound to a metabolic transformation of the hormones.

In some instances it appears that invertebrates are able to metabolize thyroid compounds in much the same manner as the tissues of vertebrates do. The hepatopancreas of the mollusks, *Mytilus galloprovincialis* and *Octopus vulgaris,* has been shown to degrade T_3 by deiodination and oxidative deamination, followed by oxidative decarboxylation (Covelli *et al.,* 1960). This is considered to be a metabolic degradation of the hormone rather than a physiological activation. The formation of thyroxine metabolites by *Escherichia coli* has also been reported (Gräsbeck *et al.,* 1960) and explained on the basis that both *E. coli* and other micro-organisms are able to oxidatively deaminate many amino acids.

The question of extrathyroidal iodine metabolism is closely related to the problem of invertebrate tissue responses to thyroid hormones. The discovery that thyroxine could be recovered from iodinated casein (Ludwig and von Mutzenbecher, 1939) was followed by early reports (Chapman, 1941; Morton *et al.,* 1943) that newly-formed thyroxine-like compounds could be demonstrated in the tissues of thyroidectomized rats. The observation that the same limited series of iodine compounds is formed when any of a large number of proteins is iodinated, whether in the thyroid, in artificially iodinated proteins, or in the iodoproteins of invertebrates (Reineke, 1949) appeared to confirm this idea, but it has recently been discredited (Taurog *et al.,* 1960) on the basis that the concentrations of iodine used in the early experiments were far above the physiological range.

The evidence to date strongly indicates that tissues of invertebrates are insensitive to the action of thyroxine and its analogs. The negative results obtained in the present experiment support this view. However, the continual recurrence of reports which substantiate the opposite view to some extent, such as the augmentation of oxygen consumption in protistans, noted above, prevents the complete acceptance of the view that invertebrate tissues are wholly insensitive to thyroid compounds. Since most reports of a positive response of non-chordate tissues to thyroxine and its analogs are limited to those experiments in which groups of like cells are used, either as tissues from metazoans or as concentrated groups of one-celled organisms, it is possible the elucidation of the cellular metabolism of thyroxine may bring to light principles which will aid in solving the question of regulation of metabolic processes in thyroidless organisms. It seems reasonable to suppose that the same fundamental pattern of metabolic regulation may be found throughout the animal kingdom.

Summary and Conclusions

1. Using the Latin square method, a study was made of the effect of the thyroid compounds diiodotyrosine, triiodothyronine, and thyroxine on respiration in planarians. No statistically significant effect was found with any one of the three chemicals under the conditions of the experiment.

2. The regulation of metabolic processes in invertebrates is discussed briefly. The suggestion is made that the same fundamental pattern of metabolic regulation may be found throughout the animal kingdom, despite the fact that no evidence of this is demonstrated in the present paper.

LITERATURE CITED

Castle, W. A., 1928. An experimental and histological study of the life cycle of *Planaria velata. J. Exp. Zool.,* **51**: 417–482.

Chapman, A., 1941. Extrathyroidal iodine metabolism. *Endocrinol.*, **29**: 686–694.

Covelli, I., F. Blasi and A. Tommaselli, 1960. Sur la dégradation de la 3:5:3′-triiodo-L-thyronine par l'hépatopancréas de la Moule (*Mytilus galloprovincialis* L.) et du Poulpe (*Octopus vulgaris* L.). *C. R. Soc. Biol.*, **154**: 1225–1228.

Goldsmith, E. D., 1937. The relation of endocrine feeding to regeneration, growth and egg capsule production in *Planaria maculata*. *Biol. Bull.*, **73**: 227–236.

Goldsmith, E. D., 1949. Phylogeny of the thyroid: Descriptive and experimental. *Ann. New York Acad. Sci.*, **50**: 283–316.

Gorbman, A., M. Clements and R. O'Brien, 1954. Utilization of radioiodine by invertebrates, with special study of several Annelida and Mollusca. *J. Exp. Zool.*, **127**: 75–92.

Gräsbeck, R., B.-A. Lamberg and F. Björkstén, 1960. The formation of thyroxine metabolites by *Escherichia coli*. *Acta Endocrinol.*, **34**: 113–120.

Gutenstein, M., and W. Marx, 1957. Stimulation of yeast respiration by L-thyroxine. *J. Biol. Chem.*, **229**: 599–602.

Jenkins, M. M., 1961. Respiration rates in planarians. II. The effect of goitrogens on oxygen consumption. *Biol. Bull.*, **120**: 370–374.

Limpel, L. E., and J. E. Casida, 1957. Iodine metabolism in insects. I. *In vivo* metabolism of radioiodide. *J. Exp. Zool.*, **135**: 19–27.

Ludwig, W., and P. von Mutzenbecher, 1939. Die Darstellung von Thyroxin, Monojodtyrosin aus jodiertem Eiweiss. *Zeitschr. physiol. Chem.*, **258**: 195–211.

Morton, M. E., I. L. Chaikoff, W. O. Reinhardt and E. M. Anderson, 1943. Radioactive iodine as an indicator of the metabolism of iodine. VI. Formation of thyroxine and diiodotyrosine by the completely thyroidectomized animal. *J. Biol. Chem.*, **147**: 757–769.

Reineke, E. P., 1949. The formation of thyroxine in iodinated proteins. *Ann. New York Acad. Sci.*, **50**: 450–465.

Roche, J., 1952. Biochimie comparée des scléroprotéines iodées des Anthozoaires et des Spongiaires. *Experientia*, **8**: 45–54.

Roche, J., G. Salvatore and I. Covelli, 1959. Sur le métabolisme der hormones thyroïdiennes par *Escherichia coli*. *C. R. Soc. Biol.*, **153**: 1116–1119.

Srinivasan, V., N. R. Moudgal and P. S. Sarma, 1955. Influence of thyroxine and thyroglobulin on rice moth larva. *Science*, **122**: 644–645.

Taurog, A., E. S. Evans, G. D. Potter and I. L. Chaikoff, 1960. Failure to demonstrate extrathyroidal thyroxine formation in thyroidectomized rats on low and moderate iodine intakes. *Endocrinol.*, **67**: 635–649.

Umbreit, W. W., R. H. Burris and J. F. Stauffer, 1949. The Warburg constant volume respirometer. *In*: Manometric Techniques and Tissue Metabolism. Minneapolis, Burgess Pub. Co.

Weimer, B. R., C. W. Phillips and D. M. Andersen, 1938. The influence of thyroxine on eye formation in *Phagocata gracilis* (Haldeman). *Physiol. Zoöl.*, **11**: 158–167.

Wingo, W. J., and L. E. Cameron, 1952. Effect of thyroxine upon growth and oxygen uptake of *Tetrahymena geleii* (Y). *Texas Rep. Biol. Med.*, **10**: 1075–1083.